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S/N 09/521,524 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Beverly L. Davidson et al.	Examiner: Shanon Foley
Serial No.:	09/521,524	Group Art Unit: 1648
Filed:	March 8, 2000	Docket: 875.025US1
Title:	RAPID GENERATION OF RECOMBINANT ADENOVIRAL VECTORS	

DECLARATION OF RICHARD D. ANDERSON AND RONALD E. HASKELL UNDER 37 C.F.R. § 1.132

1. We, Richard D. Anderson and Ronald E. Haskell, are two of the co-inventors of the above-identified patent application.

2. I, Richard D. Anderson, am a co-founder and currently the President of ViraQuest, Inc., 310 West Zeller, North Liberty, Iowa, 52317. I obtained a B.S. degree from Briar Cliff College, Sioux City, Iowa in 1985 and a B.S. (CLS) from the University of Iowa College of Medicine, Iowa City, Iowa in 1986. From 1987 through 2001, I worked as a medical researcher at the University of Iowa. From 1994-2001, I supervised the Gene Transfer Vector Core facility at the University of Iowa College of Medicine. Under my supervision the Vector Core facility produced over 300 adenoviral constructs, the subject matter of which have been published in numerous peer-reviewed scientific journals. I have collaborated with Dr. Ronald E. Haskell for seven years in the generation of recombinant adenovirus.

3. I, Ronald E. Haskell, Ph.D., am a co-founder and currently the Vice President of ViraQuest, Inc., 310 West Zeller, North Liberty, Iowa, 52317. I obtained my Ph.D. degree from Colorado State University, Fort Collins, Colorado, in 1995. Between 1995-1998, I was employed as a postdoctoral fellow, and from 1998-2001 as a Research Investigator at the University of Iowa. During this time at the University of Iowa, I performed extensive experiments using adenoviral vectors. This work has been published in five articles in peer-reviewed scientific journals. As mentioned above, I have

collaborated with Richard D. Anderson for seven years in the generation of recombinant adenovirus.

4. The above-identified application describes a cloning system for the rapid generation of recombinant adenoviral vectors. Recombinant adenoviral (Ad) vectors are frequently used to deliver cloned DNA into cells or animals, *e.g.*, *in vitro* gene transfer, *in vivo* vaccination and gene therapy (see, for example, Davidson and Roessler, Adenoviral-Mediated Gene Transfer: Potential Therapeutic Applications," Chapter 11 in Viral Vectors: Gene Therapy and Neuroscience Applications, Kaplitt and Loewy, *eds.*, Academic Press, San Diego (1995)(a copy is attached hereto as **Exhibit A**). Adenovirus type 5 (Ad5) has been used extensively for the production of recombinant adenoviral vectors (see, for example, Kozarksy and Wilson, Current Opinion in Genetics and Development, 3, 499-503 (1993) (a copy is attached hereto as **Exhibit B**). The adenovirus genome consists of linear, 36 Kb double-stranded DNA. By convention, the 36 Kb adenoviral genome is divided into 100 map units (m.u.), *i.e.*, 10 m.u. equals 3600 bp. The adenoviral genome has been completely sequenced (see GenBank accession no. M73260 (Ad5)) and well characterized. For example, the adenoviral genome contains inverted terminal repeats (ITRs) at each end, and the adenoviral gene products are organized into early (E1 through E4) and late (L1 through L5) regions. In addition, it is known that the 0-1 m.u. region contains the left hand ITR (1-103 bp) and packaging signals (194-358 bp) of the adenoviral genome. The E1 region is known to contain two genes, specifically E1A, located at 468-1676 bp, and E1B, located at 2016-3503 bp. The E3 region is known to be located at 27609-30864 bp.

5. dl309 is a biologically selected, restriction enzyme-site-loss variant of wild-type Ad5 (see Jones and Shenk, Cell, 17, 683-689 (1979) (attached hereto as **Exhibit C**). It is identical to Ad5 except for the changes reported by Jones and Shenk (1979), and as shown in GenBank accession no. U22898 (attached hereto as **Exhibit D**), *i.e.*, dl309 is

approximately 36 Kb in size. The Ad5 backbone vectors of the above-identified application are based upon dl309 (see page 8, line 21 of the specification).

6. Figure 1 of the above-identified specification illustrates the generation of pacAd5 9.2-100, an exemplary backbone plasmid of the invention. It is clear from Figure 1 that pacAd5 9.2-100 has a polyadenylation site (abbreviated as "pA" in the Figure), the 9.2-100 m.u. of Ad5 genomic DNA, a PacI restriction site that flanks the 5′ end of the pA signal, a PacI restriction site that flanks the 3′ end of the Ad5 genomic DNA, and additional plasmid DNA that contains an ampicillin resistance gene (abbreviated as "Amp" in the Figure 1) with a ScaI site. Many suitable plasmids were well-known in the art, such as pBR322 (4.3 Kb) and pUC19 (2.7 Kb). Therefore, it would be clear to that art worker that a backbone plasmid of the invention includes approximately 33 Kb of Ad5 genomic DNA and about 3-4 Kb of plasmid DNA.

7. Thus, one of ordinary skill in the art, at the time the application was filed, would have been aware that the backbone plasmids of the invention were approximately 36 Kb.

8. Standard shuttle plasmids, *i.e.*, those routinely used to co-transfect HEK293 cells, are useful in the generation of recombinant adenovirus using the system of the present invention (see page 6, lines 29-30 and page 8, lines 6-7 of the above-identified application). At the time the above-identified application was filed, there were many standard shuttle plasmids well-known in the field of recombinant adenovirus generation. Several shuttle plasmids based upon the pAdBglII shuttle plasmid were commonly employed and reported in the literature prior to the filing date of the present application (see, for example, Davidson et al., Nat. Genetics, 3, 219-223 (1993), Davidson et al., Experimental Neurology, 125, 258-267 (1994) and Aoki et al., Molecular Medicine, 5, 224-231 (1999)). When empty, *i.e.*, devoid of a subcloned cDNA of interest, pAdBglII is approximately 5.8 Kb. A copy of a cartoon depicting pAdBglII is attached (**Exhibit E**).

9. Additional examples of shuttle plasmids that were known as of the filing date of the present application and that are useful in the production of recombinant adenovirus are found in U.S. Patent 5,922,576 (He et al.; of record; referred to as "the '576 patent"). Figure 2 of the '576 patent discloses four shuttle plasmids that range in size from approximately 6.7 Kb to 9.2 Kb (when empty).

10. The function of a shuttle plasmid is to transfer cDNAs of various sizes from the shuttle vector into a viral construct (see, for example, page 6, lines 19-20 of the specification). Due to the packaging capacity of adenovirus, the range of cDNAs that can be inserted into the recombinant adenovirus using the cloning system of the invention is 0-10,000 bp.

11. Therefore, the art worker, at the time the present application was filed, would have been aware that shuttle plasmids useful in the present invention could range in size from approximately 6 Kb to approximately 20 Kb.

12. We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements are made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

7/11/03
Date

Richard D. Anderson

7/11/03
Date

Ronald E. Haskell